Determination of Anaphylatoxin Concentrations in Suction Blisters in Patients with Psoriasis

Kyoto Ohkohchi, M.D., Hideaki Takematsu, M.D., and Hachiro Tagami, M.D.
Department of Dermatology, Tohoku University School of Medicine, Sendai, Japan

Concentrations of C3a and C4a anaphylatoxins in suction blister fluids were determined by radioimmunoassay in patients with psoriasis and normal controls. Comparison of anaphylatoxin levels between serum samples and blister contents in the same subjects revealed that the levels of both C3a and C4a anaphylatoxins were significantly higher in the former than the latter even in those raised on normal skin, suggesting that the classic complement pathway is activated during suction procedure. Therefore we cannot regard suction blister fluid to be simply representative of undisturbed interstitial tissue fluid as far as the complement system is concerned.

There was no difference in anaphylatoxin levels between those from uninvolved skin of psoriatic patients and those from normal controls. However, significantly high anaphylatoxin levels were noted in fluids of suction blisters raised on lesional skin as compared with those produced on uninvolved skin in psoriatic patients. *J Invest Dermatol 87:65–67, 1986*

Identification of various chemical mediators is indispensible for the elucidation of the mechanisms underlying the development of inflammation. For the analysis of earlier inflammatory events we can use scale extracts which retain responsible chemical mediators [1]. On the other hand, to analyze active substances that take part in an ongoing inflammatory process, we have had to use freshly obtained biopsy samples or skin perfusate fluid collected after intracutaneous injection of innocuous fluid [2]. In contrast to these invasive methods, raising a suction blister on the skin surface by applying negative pressure which produces dermal-epidermal separation at the level of the lamina lucida has become a popular method for collecting tissue fluid, as this technique is regarded a far less damaging to the tissues than other methods [2,3]; the blister fluid has been accepted to be ideally representative of interstitial fluid [4,5].

In characteristic psoriatic lesions there occurs cyclic transepidermal leukocyte migration [6]. We demonstrated the presence of specific chemotactic peptide(s) in psoriatic scale which is thought to play a key role in the development of this phenomenon, and obtained evidence that it contains complement-derived chemotactic factor [1]. In addition, leukotriene B_4, the most potent lipid chemoattractant of 5-lipoxygenase product, was also identified in chamber fluid of psoriatic lesions [7] or in psoriatic scale extracts [8,9], which could function along with chemotactic anaphylatoxin to induce neutrophil migration into psoriatic epidermis. Recently we have found increased amounts of C5 cleavage fragments (either C5a or $C5a_{des\ arg}$) in psoriatic scale extracts together with other anaphylatoxins, C3a and C4a [10]. They are also increased in sera of psoriatic patients [11]. These findings suggest the occurrence of classical complement pathway activation in psoriatic patients.

In the present study, to obtain more direct information about the complement system in lesional skin, we have measured C3a and C4a anaphylatoxin levels in suction blister contents raised in psoriatic lesions. However, we have found that complement activation takes place probably through the classical pathway during the production procedure of suction blister even in normal skin.

MATERIALS AND METHODS

Subjects Sixteen psoriatic patients (12 males and 4 females, ranging in age from 19–82 years with a mean of 49 years) and 12 healthy volunteers (11 males and 1 female aged 10–34 years with a mean age of 29 years) were studied. None of the patients had received any kind of treatment for at least 2 weeks before examination.

Suction Blister Formation Blisters were produced on the skin of the abdomen with an attachment device using a hollow syringe-cylinder after removing a piston from a 2-ml disposable syringe (Jintan Pharmacological Co., Tokyo), whose broad and flat end was placed on the skin and a negative pressure of 200 mm Hg was applied from a rubber suction tube connected to a nozzle portion of the syringe. Suction blisters could be raised even in lesional skin within a few hours in the same way as on normal skin if the lesional skin was not covered with thick scales. There was no appreciable difference in required suction time between involved and uninvolved skin. If the collected amount was not sufficient for the measurement of the 2 anaphylatoxin levels, at least one anaphylatoxin level was determined.

In subjects in whom a comparison of the anaphylatoxin levels between serum and suction blister fluid was performed, the blood samples were also obtained at the termination of suction. The collected blister fluids and freshly separated serum samples were stored in the presence of EDTA at −70°C as described previously [8].

Radioimmunoassay Radioimmunoassays for human C3a and $C3a_{des\ arg}$, C4a and $C4a_{des\ arg}$, referred to here collectively as C3a and C4a, were performed as reported previously [10–12]. The assay kits for $C3a_{des\ arg}$ and $C4a_{des\ arg}$ were obtained from Upjohn Diagnostics (Kalamazoo, Michigan) and the assays were carried out according to the manufacturer's instructions.

Statistical Analysis Means and standard deviations were calculated and shown. Levels of anaphylatoxins were compared using Student's *t*-test or the Wilcoxon rank-sum test.

Manuscript received October 3, 1985; accepted for publication January 30, 1986.

Supported by Grants-in-Aid for Scientific Research 5940244 from the Ministry of Education, Science and Culture, Japan.

Reprint requests to: Hachiro Tagami, M.D., Department of Dermatology, Tohoku University School of Medicine, 1-1 Seiryo-machi, Sendai 980, Japan.

0022-202X/86/$03.50

RESULTS

Comparison of Anaphylatoxin Levels Between Serum and Fluid from Suction Blister Raised on Normal Skin The effect of suction of normal skin to produce dermal-epidermal separation on anaphylatoxin levels was investigated by comparing C3a and C4a anaphylatoxin levels between serum and suction blister fluid in the same individuals consisting of 2 control subjects and 3 psoriatic patients. The anaphylatoxin levels in suction blister fluids were found to be 3–14 times more than those of the respective serum samples (Fig 1). Hence, the mean values of both C3a and C4a levels in blister fluids were significantly higher than those of the serum levels ($p < 0.001$).

There was no correlation between the levels of serum samples and those of blister contents, suggesting that anaphylatoxins are produced in the skin independent of their serum concentrations.

Anaphylatoxin Levels in Suction Blisters Produced in Psoriatic Lesions The mean values of C3a and C4a in the blister fluids obtained from uninvolved paralesional skin of psoriatic patients were 981 ± 693 ng/ml (n = 13) and 869 ± 416 ng/ml (n = 16), respectively, neither of which differed significantly from the corresponding values noted in blisters raised in normal subjects, i.e., 940 ± 259 ng/ml (n = 10) and 739 ± 322 ng/ml (n = 10) (Fig 2).

In contrast, significantly high anaphylatoxin concentrations were noted in the blister contents obtained from lesional skin, i.e., 1986 ± 1099 ng/ml for C3a (n = 14) ($p < 0.005$ against those of normal controls and $p < 0.025$ against those of uninvolved psoriatic skin) and 1365 ± 604 ng/ml for C4a (n = 14) ($p < 0.005$ against those of normal controls and $p < 0.025$ against those of uninvolved psoriatic skin). When a comparison was made in 5 patients in whom all the data of serum and blister fluids taken from both uninvolved and involved skin were available, these differences became much more apparent (data not shown).

DISCUSSION

The present study was designed to extend our previous investigation about elevated levels of both C3a and C4a anaphylatoxins in psoriatic lesional scales [10] which suggested a preceding event involving the classical complement pathway activation in the lesional skin. We also found definitely higher serum anaphylatoxin concentrations in psoriatic patients [11]. If suction blisters simply contain leaked serum components by passive diffusion [4], their anaphylatoxin levels should reflect the serum levels. However, we found that the levels of anaphylatoxins in blister contents did not correlate well with those of the corresponding serum levels but that their levels were much higher in suction blister fluids than in sera.

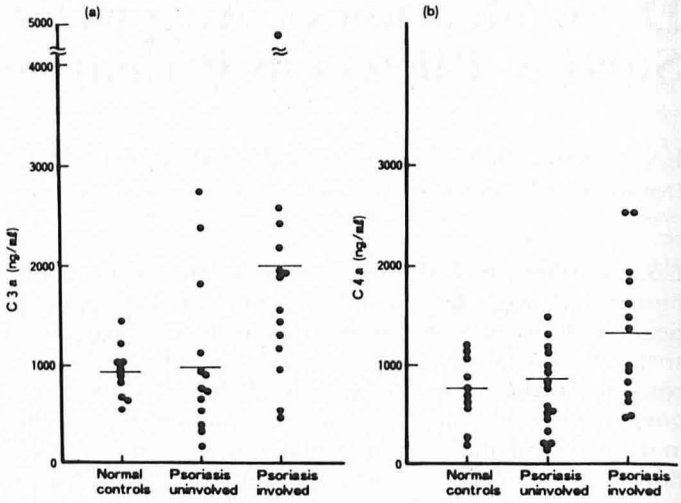

Figure 2. Levels of C3a (*a*) and C4a (*b*) anaphylatoxins in suction blisters raised in normal subjects and those in blisters produced in uninvolved and involved skin of psoriatic patients. *Bars* represent mean values.

In the present investigation we used serum instead of plasma which is conventionally used as a sample for radioimmunoassay of complement split products in the blood [12]. The reason was that in our former study [11] we used serum instead of plasma to determine the blood anaphylatoxin concentrations; in our preliminary study, we did not find any significant difference in anaphylatoxin levels between serum and plasma when they were compared in the same subjects. Therefore, the present results clearly indicate that the anaphylatoxin levels in suction blister fluids are remarkably higher than those in the blood.

Clemmensen et al [13] found a minor increase in C3-split in suction blister fluid taken from normal skin by crossed immunoelectrophoresis. Our present study has provided definite evidence for the complement activation taking place during the production of suction blisters.

Detection of increases in both C4a and C3a levels suggests the classical complement pathway activation. The procedure to raise a suction blister on the skin has been accepted as a rather noninvasive way for obtaining chemical mediators present in the tissue fluid [2]. However, it is now clear that, as far as the complement system is concerned, the blister fluid is not a simple transudate from the peripheral blood. This is a distinct difference from the case of other serum proteins or chemical mediators of inflammation [2,5,7,14]. We think that forced accumulation of tissue fluid in a certain portion of the skin by applying negative pressure may increase the chance for serum complement components to be exposed to usually hidden tissue components which have a property to activate the classical complement pathway.

Several possible sources are suggested for the high levels of anaphylatoxins in suction blister contents from lesional skin. In addition to those anaphylatoxins that have already been produced in situ before the production of suction blisters, there is a possibility that large amounts of fresh anaphylatoxins are produced during the formation of suction blisters. Since even normal skin components activate complement during the production of suction blisters, it is quite possible that complement activation occurs to a much larger extent during suction blister formation in psoriatic lesions which are rich in infiltrating cells and inflammatory exudate with enhanced levels of various proteinases [15]. In in-

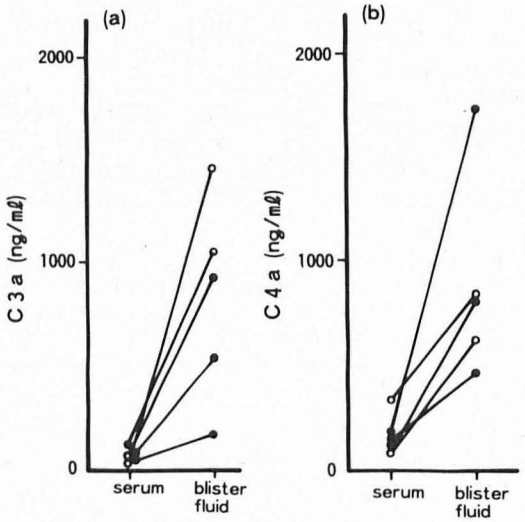

Figure 1. Comparison of C3a (*a*) and C4a (*b*) anaphylatoxin levels between serum and suction blister content in the same individuals. *Open circles* represent the values of healthy volunteers and *closed circles* those of psoriatic patients.

volved psoriatic tissue there are both enhanced levels of serine proteinase [16] and polymorphonuclear leukocytes which have the capability to directly cleave C5 to produce chemotactic C5a anaphylatoxin and to activate both the alternative and classic pathways [17,18]. However it remains obscure exactly what tissue components in psoriatic lesions are responsible for the production of C3a and C4a anaphylatoxins in suction blisters. Furthermore, it is possible that antigen–antibody complexes in the skin of psoriatic patients [19,20] may activate the complement system.

In conclusion, the procedure to raise suction blisters on the skin induces classic complement pathway activation. Therefore, although there may be increased amounts of anaphylatoxins in psoriatic lesional skin, it is difficult to determine the exact in situ concentrations of C3a and C4a anaphylatoxins from the analysis of the suction blister contents.

REFERENCES

1. Tagami H, Ofuji S: Characterization of leukotactic factor derived from psoriatic scale. Br J Dermatol 97:509–518, 1977
2. Black AK, Greaves MW, Hensby CN, Plummer NA, Eady RAJ: A new method for recovery of exudates from normal and inflamed human skin. Clin Exp Dermatol 2:209–216, 1977
3. Greaves MW, Black AK: Identification of mediators of inflammation, Investigative Techniques in Dermatology. Edited by R Marks. Oxford, Blackwell Scientific, 1979, pp 94–103
4. Kiistala U: Suction blister device for separation of viable epidermis from dermis. J Invest Dermatol 50:129–137, 1968
5. Vermeer BJ, Reman FC, van Gent CM: The determination of lipids and proteins in suction blister fluid. J Invest Dermatol 73:303–305, 1979
6. Pinkus H, Mehregan AM: The primary lesion in seborrheic dermatitis and psoriasis. J Invest Dermatol 46:109–115, 1966
7. Brain SD, Camp RDR, Dowd PM, Black AK, Woollard PM, Mallet AI, Greaves MW: Psoriasis and leukotriene B_4. Lancet 2:762, 1982
8. Grabbe J, Czarnetzki BM, Rosenbach T, Mardin M: Identification of chemotactic lipoxygenase products of arachidonate metabolism in psoriatic skin. J Invest Dermatol 82:477–479, 1984
9. Takematsu H, Terui T, Tagami H: Demonstration of leukotriene B_4 in the scale extracts of psoriasis and inflammatory pustular dermatoses. Correlation with leukocyte chemotactic activity and C5a anaphylatoxin. Acta Derm Venereol (Stockh) 66:6–10, 1986
10. Takematsu H, Ohkohchi K, Tagami H: Demonstration of anaphylatoxins C3a, C4a, and C5a in the scales of psoriasis and inflammatory pustular dermatoses. Br J Dermatol 114:1–6, 1986
11. Ohkohchi K, Takematsu H, Tagami H: Increased C3a and C4a anaphylatoxins in psoriatic sera. Br J Dermatol 113:189–196, 1985
12. Hugli TE, Chenoweth DE: Biologically active peptides of complement: techniques and significance of C3a and C5a measurements, Immunoassays: Clinical Laboratory Techniques for the 1980s. Edited by RM Nakamura. New York, Alan R Liss, 1980, pp 443–460
13. Clemmensen OJ, Staberg B, Worm A-M: Activation of complement in the skin after PUVA therapy. Acta Derm Venereol (Stockh) 64:5–8, 1984
14. Barr RM, Brain S, Camp RDR, Cillers J, Greaves MW, Mallet AI, Misch K: Levels of arachidonic acid and its metabolites in the skin in human allergic and irritant contact dermatitis. Br J Dermatol 111:23–28, 1984
15. Fraki JE, Hopsu-Havu VK: Human skin proteases: fractionation of psoriasis scale proteases and separation of plasminogen activator and a histone hydrolyzing protease. Arch Dermatol Res 2256:113–126, 1976
16. Lazarus GS, Yost FJ, Thomas CA: Polymorphonuclear leukocytes: possible mechanism of accumulation in psoriasis. Science 198:1162–1164, 1977
17. Glinski W, Braszcz D, Janczura E, Zarebska Z, Jablonska S: Neutral proteinases and other neutrophil enzymes in psoriasis, and their relation to disease activity. Br J Dermatol 111:147–154, 1984
18. Wright DG, Gallin JI: Modulation of the inflammatory response by products released from human polymorphonuclear leukocytes during phagocytosis. Inflammation 1:23–39, 1975
19. Beutner EH, Jarzabek-Chorzelska M, Jablonska S, Chorzelski TP, Rzesa G: Autoimmunity in psoriasis. A complement immunofluorescence study. Arch Dermatol Res 261:123–134, 1978
20. Ullman S, Halberg P, Nentzer B: Deposits of immunoglobulin and complement in psoriatic lesions. J Cutan Pathol 7:271–275, 1980